Chapter 20
Electron Transport and Oxidative Phosphorylation

SUMMARY

Section 20.1

- Electron transport from one carrier to another creates a proton gradient across the inner mitochondrial membrane.
- The proton gradient is coupled to the production of ATP in aerobic metabolism.

Section 20.2

- Standard reduction potentials provide a basis for comparison among oxidation-reduction reactions.
- The sequence of reactions in the electron transport chain can be predicted by using reduction potentials.

Section 20.3

- The electron transport chain consists of four multisubunit membrane-bound complexes and two mobile electron carriers (coenzyme Q and cytochrome c). The reactions that take place in three of these complexes generate enough energy to drive the phosphorylation of ADP to ATP.
- Many protein of the electron-transport chain contain iron, either as part of a heme or combined with sulfur.

Section 20.4

- The coupling of electron transport to oxidative phosphorylation requires a multisubunit membrane- bound enzyme, ATP synthase. This enzyme has a channel for protons to flow from the intermembrane space into the mitochondrial matrix.
- The proton flow is coupled to ATP production in a process that appears to involve a conformational change of the enzyme.

Section 20.5

- In chemiosmotic coupling, the proton gradient is the crux of the matter. The flow of protons through a pore in the synthase drives ATP production.
- In conformational coupling, a change in the shape of the synthase releases bound ATP that has already been formed.

Section 20.6

- Shuttle mechanisms transfer electrons, but not NADH, from the cytosol across the mitochondrial membrane.
- In the malate–aspartate shuttle, 2.5 molecules of ATP are produced for each molecule of cytosolic NADH, rather than 1.5 ATP in the glycerol–phosphate shuttle, a point that affects the overall yield of ATP in these tissues.

Section 20.7

- In the complete oxidation of glucose, a total of 30 or 32 molecules of ATP are produced for each molecule of glucose, depending on the shuttle mechanism.

LECTURE NOTES

As is the case for much of central energy metabolism, students are likely to be at least passingly familiar with the basics, but none of the details covered by this chapter. A review of electrochemistry helps connect the underlying energetics to other metabolic pathways. The major focus is on the proton gradient that couples the two processes of electron transport and oxidative phosphorylation. This chapter will likely require two lectures for complete coverage of the material

LECTURE OUTLINE

I. Role of electron transport in metabolism
    A. Production of proton gradient
    B. Coupling to oxidative phosphorylation
    C. Central nature of coenzyme Q
II. Reduction Potentials
    A. Connection to ΔG
    B. Reference and half-cells
    C. Nernst equation
III. Organization of electron transport complexes
    A. Complex I
        1. Transfer of electrons from NADH to CoQ
        2. Iron-sulfur clusters
        3. Flavin mononucleotide structure and function
        4. Coenzyme Q structure and function
        5. Contribution to proton gradient
    B. Complex II
        1. Identity as succinate dehydrogenase
        2. Covalently bound FAD
        3. Iron-sulfur clusters and their redox states
        4. No protons pumped
    C. Complex III
        1. Transfer of electrons from CoQ to cytochrome c
        2. Connection between two-electron and one-electron carriers
        3. Cytochromes bL, bH, and c1
        4. Q-cycle
        5. Proton pumping
    D. Complex IV
        1. Electron transfer from cytochrome c to oxygen
        2. Proton pumping
        3. Involvement of copper, cytochromes a and a3
    E. Details of cytochromes and other iron-containing proteins
IV. Connection between electron transport and phosphorylation
    A. Electrochemical potential of proton gradient
    B. ATP synthase structure
    C. Uncouplers
    D. P/O ratios
        1. NADH = 2.5

2. FADH2 = 1.5

V. Mechanism of coupling
   A. Chemiosmotic coupling — experimental evidence
   B. Conformational aspects

VI. Shuttle mechanisms
   A. Glycerol-phosphate shuttle
   B. Malate-aspartate shuttle

VII. ATP yield from complete oxidation of glucose

ANSWERS TO PROBLEMS

20.1 The Role of Electron Transport in Metabolism

1. Electrons are passed from NADH to a flavin-containing protein to coenzyme Q. From coenzyme Q, the electrons pass to cytochrome *b,* then to cytochrome *c,* via the Q cycle, followed by cytochromes *a* and a_3. From the cytochrome aa_3 complex, the electrons are finally passed to oxygen.
2. Electron transport and oxidative phosphorylation are different processes. Electron transport requires the respiratory complexes of the inner mitochondrial membrane, whereas oxidative phosphorylation requires ATP synthase, also located on the inner mitochondrial membrane. Electron transport can take place in the absence of oxidative phosphorylation.
3. In all reactions, electrons are passed from the reduced form of one reactant to the oxidized form of the next reactant in the chain. The notation [Fe—S] refers to any one of a number of iron–sulfur proteins.

Reactions of Complex I

$NADH + E\text{-}FMN \rightarrow NAD^+ + E\text{-}FMNH_2$

$E\text{-}FMNH_2 + 2[Fe\text{—}S]_{ox} \rightarrow E\text{-}FMN + 2[Fe\text{—}S]_{red}$

Liberation of enough energy to produce ATP

Transfer to Coenzyme Q

$2[Fe\text{—}S]_{red} + CoQ \rightarrow 2[Fe\text{—}S]_{ox} + CoQH_2$

Reactions of Complex III

Q cycle reactions

$[Fe\text{—}S]_{red} + cyt\ c_{1ox} \rightarrow [Fe\text{—}S]_{ox} + cyt\ c_{1red}$

Liberation of enough energy to produce ATP

Transfer to cytochrome *c*

$cyt\ c_{2red} + cyt\ c_{ox} \rightarrow cyt\ c_{1ox} + cyt\ c_{red}$

Reactions of Complex IV

$cyt\ c_{red} + cyt\ a\text{-}a_{3ox} \rightarrow cyt\ c_{ox} + cyt\ a\text{-}a_{3red}$

$cyt\ a\text{-}a_{3red} + \frac{1}{2}O_2 \rightarrow cyt\ a\text{-}a_{3ox} + H_2O$

Liberation of enough energy to produce ATP

4. When $FADH_2$ is the starting point for electron transport, electrons are passed from $FADH_2$ to coenzyme Q in a reaction carried out by Complex II that bypasses Complex I.

   $FADH_2 + 2[Fe\text{—}S]_{ox} \rightarrow FAD + 2[Fe\text{—}S]_{red}$

   $2[Fe\text{—}S]_{red} + CoQ \rightarrow 2[Fe\text{—}S]_{ox} + CoQH_2$
5. Mitochondrial structure confines the reduced electron carriers produced by the citric acid cycle to the matrix. There they are close to the respiratory complexes of the electron transport chain that will pass the electrons from the carriers produced by the citric acid cycle to oxygen, the ultimate recipient of electrons and hydrogens.

20.2 Reduction Potentials in the Electron Transport Chain

6. The electron transport chain translocates charged particles by chemical means. Interconversion of chemical and electrical energy is exactly what a battery does.
7. The reactions are all written in the same direction for purposes of comparison. By convention, they are written as reduction, rather than oxidation, reactions.
8. $\Delta G^{\circ\prime}$ = –60 kJ/mol
9. We fundamentally add the half reactions in Table 20.1.

	$E^{\circ\prime}$ (V)
$NAD^+ + 2H^+ + 2e^- \rightarrow NADH + H^+$	−0.320

This is the wrong direction, so we reverse the equation and the sign of the potential difference.

$NADH + H^+ \rightarrow NAD^+ + 2H^+ + 2e^-$	0.820
$\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O$	0.816
$NADH + H^+ + \frac{1}{2}O_2 \rightarrow NAD^+ + H_2O$	1.136

10. We fundamentally add the half reactions in Table 20.1.

	$E^{\circ\prime}$ (V)
$NAD^+ + 2H^+ + 2e^- \rightarrow NADH + H^+$	−0.320

This is the wrong direction, so we reverse the equation and the sign of the potential difference.

$NADH + H^+ \rightarrow NAD^+ + 2H^+ + 2e^-$	0.820
$Pyruvate + 2H^+ + 2e^- \rightarrow Lactate$	−0.185
$NADH + H^+ + Pyruvate \rightarrow NAD^+ + Lactate$	0.135

11. We fundamentally add the half reactions in Table 20.1.

	$E^{\circ\prime}$ (V)
$Fumarate + 2H^+ + 2e^- \rightarrow Succinate$	0.031

This is the wrong direction, so we reverse the equation and the sign of the potential difference.

$Succinate \rightarrow Fumarate + 2H^+ + 2e^-$	−0.031
$\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O$	0.816
$Succunate + \frac{1}{2}O_2 \rightarrow Fumarate + H_2O$	0.785

12. The cytochrome is the electron donor, and the flavin moiety is the electron acceptor. Once again, we add the half reactions in Table 20.1.

	$E^{\circ\prime}$ (V)
Cytochrome $c(Fe^{3+}) + e^- \rightarrow$ Cytochrome $c(Fe^{2+})$	0.254

This is the wrong direction, so we reverse the equation and the sign of the potential difference.

2 Cytochrome $c(Fe^{2+}) + 2e^- \rightarrow$ 2 Cytochrome $c(Fe^{3+})$	−0.254
$[FAD] + 2\,H^+ + 2\,e^- \rightarrow [FADH_2]$	0.091
$[FAD] + 2$ Cyt $c(Fe^{2+}) + 2H^+ \rightarrow [FADH_2] + 2$ Cyt $c(Fe^{3+})$	−0.068

This was the maximum value for a bound flavin. The negative sign indicates that this reaction will not take place as written because it is not energetically favorable.

13. Here is an illustration based on standard reduction potentials.

	$E°'$ (V)
Fumarate + $2H^+$ + $2e^-$ → Succinate	0.081

This is the wrong direction, so we reverse the equation and the sign of the potential difference.

Succinate → Fumarate + $2H^+$ + $2e^-$	−0.081
FAD + $2H^+$ + $2e^-$ → $FADH_2$	−0.219
Succinate + FAD → Fumarate + $FADH_2$	−0.250

The other possibility can be calculated in the same way.

Succinate → Fumarate + $2H^+$ + $2e^-$	−0.081
NAD^+ + $2H^+$ + $2e^-$ + NADH + H^+	0.320
Succinate + NAD^+ → Fumarate + NADH	−0.851

Both reduction potentials indicate a reaction that is not energetically favorable, but less so with FAD than with NAD^+. Other factors enter into consideration, however, in a living cell. The first is that the reactions do not take place under standard conditions, altering the values of reduction potentials. The second is that the reduced electron carriers (NADH and $FADH_2$) are reoxidized. Coupling the reactions we have looked at here to others also makes them less unfavorable.

14. The half reaction of oxidation NADH + H^+ → NAD^+ + $2H^+$ + $2e^-$ is strongly exergonic ($\Delta G°'$ = –61.3 kJ mol^{-1} = –14.8 kcal mol^{-1}), as is the overall reaction Pyruvate + NADH + H^+ → Lactate + NAD^+ ($\Delta G°'$ = –25.1 kJ mol^{-1} = –6.0 kcal mol^{-1}).

20.3 Organization of Electron Transport Complexes

15. They all contain the heme group, with minor differences in the heme side chains in most cytochromes.
16. Cytochromes are proteins of electron transport; the heme ion alternates between the Fe(II) and Fe(III) states. The function of hemoglobin and myoglobin is oxygen transport and storage, respectively. The iron remains in the Fe(II) state.
17. Coenzyme Q is not bound to any of the respiratory complexes. It moves freely in the inner mitochondrial membrane.
18. A part of Complex II catalyzes the conversion of succinate to fumarate in the citric acid cycle.
19. Three of the four respiratory complexes generate enough energy to phosphorylate ADP to ATP. Complex II is the sole exception.
20. Cytochrome *c* is not tightly bound to the mitochondrial membrane and can easily be lost in the course of cell fractionation. This protein is so similar in most aerobic organisms that cytochrome *c* from one source can easily be substituted for that from another source.

21. Succinate + ½ O_2 → Fumarate + H_2O
22. The components are in the proper orientation for the electrons to be transferred rapidly from one component to the next; if the components were in solution, speed would be limited to the rate of diffusion. A second advantage, which is actually a necessity, is that the components are properly positioned to facilitate the transport of protons from the matrix to the intermembrane space.
23. From an evolutionary standpoint, two different functions can be performed by identical structures or by structures that are close to identical, with only minor differences in the protein moieties. The organism saves a considerable amount of energy by not having to evolve—and to operate—two pathways.
24. The key point here is not the active site, which has a low tolerance for mutations, but the molecules with which the proteins in question are associated. Cytochromes are membrane-bound and must associate with other members of the electron transport chain; most mutations are likely to interfere with the close fit, and thus they are not preserved (because they are lethal). Globins, although soluble, still form some associations, so more mutations can be tolerated, with some limits. Hydrolytic enzymes are soluble and not likely to associate with other polypeptides except substrates. They can tolerate a higher proportion of mutations.
25. Having mobile electron carriers in addition to membrane-bound respiratory complexes allows electron transport to use the most readily available complex rather than to use the same one all the time.
26. The Q cycle allows for a smooth transition from two-electron carriers (NADH and $FADH_2$) to one-electron carriers (cytochromes).
27. The protein environment of the iron differs in each of the cytochromes, causing differences in the reduction potential.
28. All the reactions in the electron transport chain are electron-transfer reactions, but some of the reactants and products inherently transfer either one or two electrons, as the case may be.
29. The heme groups differ slightly in the various kinds of cytochromes. This is the main difference, with some modification due to the different protein environments.
30. Respiratory complexes contain a number of proteins, some of them quite large. This is the first difficulty. Like most proteins bound to membranes, the components of respiratory complexes are easily denatured on removal from their environment.

20.4 The Connection between Electron Transport and Phosphorylation

31. The F_1 portion of the mitochondrial ATP synthase, which projects into the matrix, is the site of ATP synthesis.
32. The F_0 portion of mitochondrial ATP synthase lies within the inner mitochondrial membrane, but the F_1 portion projects into the matrix.
33. The P/O ratio gives the number of moles of P_i consumed in the reaction ADP + P_i → ATP for each mole of oxygen atoms consumed in the reaction ½ O_2 + $2H^+$ → $2H_2O$. It is a measure of the coupling of ATP production to electron transport.

34. The F_1 part of mitochondrial ATP synthase has a stationary domain (the $\alpha_3\beta_3\delta$ domain) and a domain that rotates (the $\gamma\varepsilon$ domain). This is exactly the arrangement needed for a motor.
35. A P/O ratio of 1.5 can be expected because oxidation of succinate passes electrons to coenzyme Q via a flavoprotein intermediate, bypassing the first respiratory complex.
36. Exact values for P/O ratios are difficult to determine because of the complexity of the systems that pump protons and phosphorylate ADP. The number of ADP molecules phosphorylated is directly related to the number of protons pumped across the membrane. This figure has been a matter of some controversy. It has been difficult for chemists and biochemists to accept uncertain stoichiometry.
37. The difficulties in determining the number of protons pumped across the inner mitochondrial membrane by respiratory complexes are those inherent in working with large assemblies of proteins that must be bound in a membrane environment to be active. As experimental methods improve, the task becomes less difficult.

20.5 The Mechanism of Coupling in Oxidative Phosphorylation

38. The chemiosmotic coupling mechanism is based on the difference in hydrogen ion concentration between the intermembrane space and the matrix of actively respiring mitochondria. The hydrogen ion gradient is created by the proton pumping that accompanies the transfer of electrons. The flow of hydrogen ions back into the matrix through a channel in the ATP synthase is directly coupled to the phosphorylation of ADP.
39. An intact mitochondrial membrane is necessary for compartmentalization, which in turn is necessary for proton pumping.
40. Uncouplers overcome the proton gradient on which oxidative phosphorylation depends.
41. In chemiosmotic coupling, the proton gradient is related to ATP production. The proton gradient leads to conformational changes in a number of proteins, releasing tightly bound ATP from the synthase as a result of the conformational change.
42. Dinitrophenol is an uncoupler of oxidative phosphorylation. The rationale was to dissipate energy as heat.
43. The energy released as protons pass through the F particles is actually used to cause conformational changes in the F_1 proteins, thereby releasing ATP. The “tight” conformation (one of three) provides a hydrophobic environment in which ADP is phosphorylated by adding P_i without requiring *immediate* energy.
44. Uncouplers and respiratory inhibitors act in different ways. Uncouplers lead to increased permeability of the inner mitochondrial membrane, making oxidative phosphorylation less effective. Respiratory inhibitors block the transfer of electrons from one component of the electron transport chain to the next component.
45. Electron micrographs shoe different conformations in active and resting mitochondria.

46. Experiments with model systems have shown that electron transport and proton pumping can take place separately.

20.6 Shuttle Mechanisms

47. The complete oxidation of glucose produces 30 molecules of ATP in muscle and brain and 32 ATP in liver, heart, and kidney. The underlying reason is the different shuttle mechanisms for transfer to mitochondria of electrons from the NADH produced in the cytosol by glycolysis.
48. The transport "product" (in the matrix) of the malate–aspartate shuttle is NADH, whereas that of the glycerol–phosphate shuttle is $FADH_2$. The latter shuttle can thus go *against* a transmembrane NADH concentration gradient, whereas the former cannot.

20.7 The ATP Yield from Complete Oxidation of Glucose

49.

(a) 34
(b) 32
(c) 13.5
(d) 17
(e) 2.5
(f) 12.5

50. The maximum yield of ATP, to the nearest whole number, is 3.

$$102.3 \text{ kJ released} \times \frac{1 \text{ ATP}}{30.5 \text{ kJ}} = 3.35 \text{ ATP}$$

One ATP is actually produced, so the efficiency of the process is

$$\frac{1 \text{ ATP}}{3 \text{ ATP}} \times 100 = 33.3\%$$